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(71) Applicant: **INSTITUT NATIONAL DE LA
RECHERCHE AGRONOMIQUE (INRA)
75007 Paris Cédex 07 (FR)**

(72) Inventors:
• **Boucher, Christian
31650 Auzielle (FR)**

- **Elbaz, Mounira
2042 Tunis (TN)**
- **Genin, Stéphane
31450 Donneville (FR)**
- **Guidot, Alice
69300 Caluire (FR)**
- **Prior, Philippe
97432 Ravine des Cabris (FR)**

(74) Representative: **Colombet, Alain André et al
Cabinet Lavoix
2, Place d'Estienne d'Orves
75441 Paris Cedex 09 (FR)**

(54) **Method for detecting *ralstonia solanacearum* race 3 biovar 2**

(57) The invention concerns a method for the detection of *Ralstonia solanacearum* race 3 biovar 2 strains in a medium, comprising the determination of the presence or the absence in a sample of the medium, of:

- (i) at least one first nucleic acid target having a sequence selected from the group constituted of SEQ ID NO: 1-49, complementary sequences thereof, and homologous sequences thereof, or
- (ii) at least one fragment of said first target nucleic acid,

wherein said fragment is not constituted of or comprised in a sequence selected from the group constituted of SEQ ID NO: 111-140;

whereby, if said first nucleic acid target or fragment thereof is present in the sample, it is determined that *Ralstonia solanacearum* race 3 biovar 2 strain is present in the medium.

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Description**Field of the invention**

[0001] The present invention relates to a method for detecting race 3 biovar 2 *Ralstonia solanacearum* strains.

Background of the invention

[0002] *Ralstonia solanacearum* is a Gram-negative soil-borne plant pathogen with thousands of distinct strains in a heterogeneous species complex. *Ralstonia solanacearum* causes bacterial wilt, which is globally distributed and economically destructive. It is thus considered the single most destructive bacterial plant disease because of its unusually broad host range. The bacterium attacks plants in over 200 different families, including dicots and monocots, and annual plants as well as trees. Economically important crop hosts include: tomato, potato, pepper, tobacco, peanut, ornamentals, banana, plantain, and eucalyptus. Losses due to bacterial wilt are known to be enormous but cannot be accurately estimated because of its large but undocumented impact on subsistence agriculture and because planting of wilt-susceptible crops have been abandoned altogether in many parts of the world. Specifically, economic losses to the potato industry in the world have been estimated 950 million US \$ and the potato brown rot strain of *Ralstonia solanacearum* was listed in the USA as a Bioterrorism Select Agent (BSA).

[0003] The pathogen usually enters host roots from the soil, multiplies in the root cortex, and colonizes the xylem vessels, so the bacteria spread rapidly throughout the host via the plant's own vascular system. Symptoms vary according to host, but rapid wilting and death are the common elements.

[0004] To unravel the genetic diversity within this species complex, a new classification scheme was proposed that distinguishes four phylotypes:

- phylotype I corresponds to the "Asiaticum" division 1 of Cook et al (1989) and contains strains belonging to biovars 3 (GMI1000), 4 and 5;
- phylotype II corresponds to the "Americanum" division 2 of Cook and contains strains belonging to race 1 biovar 1, race 2 biovar 1 (e.g. Moko disease-causing strains such as Molk2), race 3 biovar 2 (e.g. IPO1609 and UW551) and race 3 biovar 2T strains;
- phylotype III contains strains from Africa and the Indian Ocean, which belong to biovars 1, 2 and 2T;
- phylotype IV is reported highly heterogeneous; it contains strains from Indonesia, some strains from Japan, and a single strain from Australia, belonging to biovars 1, 2 and 2T; phylotype IV also contains the closely related species *Ralstonia syzigii* and the blood disease bacterium (BDB).

[0005] Each phylotype can be further subdivided into sequevars based on differences in partial sequence of the endoglucanase gene (eg/). The phylotyping scheme is broadly consistent with the former phenotypic and molecular typing schemes (Fegan and Prior, 2005; Prior and Fegan 2005), and adds valuable information about the geographical origin and in some cases the pathogenicity of strains. It is believed that, after the race/biovar classification, the phylotype classification scheme is to become the core organizing principle for assigning a particular strain a phylogenetic position with a predictive value on potential host range (Fegan and Prior, 2006; Wicker et al., 2007).

[0006] Whole genome sequencing was decisive in unravelling the broad genetic diversity encompassed within this organism with unusually broad host range. Thus a metagenomic microarray from sequence data of a broad host range tomato phylotype I strain (GMI1000) has been developed which has enabled comparative genomic hybridizations demonstrating that a third of the *Ralstonia solanacearum* genome is constituted of variable genes probably acquired by horizontal gene transfers. The distribution of variable genes between strains is related to the phylotype classification (Guidot et al, 2007).

[0007] Recent phylogenetic evidences indicated that strains that fit with the definition of the potato brown rot agent were placed into the phylotype IIB sequevar 1 and 2, i.e. the biovar 2 Andean strains of *Ralstonia solanacearum* historically known as race 3 biovar 2. These strains are highly pathogenic to potato and adapted to low temperatures. Some strains in that group were also reported to carry an enlarged host range including tomato, and *Geranium rosa* (Carneille et al., 2006).

[0008] Given, the important economic impact of potato brown rot, it is highly desirable to develop methods for specifically detecting race 3 biovar 2 *Ralstonia solanacearum* strains.

[0009] Thus, WO 2004/042016 relates to real-time PCR primers and probes useful for the detection of such strains. The primers and probes enable the detection of a nucleic acid sequence which is specific for race 3 biovar 2 *Ralstonia solanacearum* strains.

[0010] However, it appears that the nucleic acid sequence detected by the primers and probes of WO 2004/042016 is that of a mobile genetic element, since this sequence encodes part of a protein homologous to the Mu-like phage of

the ORF35 of the B3 bacteriophage from *Pseudomonas aeruginosa* (Accession Q7AX27).

[0011] In general, mobile genetic elements should be avoided in the frame of the specific detection of a pathogenic microorganism, since particular strains of the pathogenic microorganism could lack the element or, conversely, other unrelated microorganisms could harbour the element, thereby yielding respectively false negative and false positive results.

[0012] Accordingly, it is an object of the present invention to develop a method for detecting race 3 biovar 2 *Ralstonia solanacearum* strains which involves the detection of specific nucleic acid sequences which do not belong to mobile genetic elements.

Description of the invention

[0013] The present invention arises from the identification, by the inventors, of genomic portions of *Ralstonia solanacearum* which are specific of the race 3 biovar 2 strains of *Ralstonia solanacearum*.

[0014] Thus the present invention relates to a method for the detection of *Ralstonia solanacearum* race 3 biovar 2 in a medium, comprising the determination of the presence or the absence in a sample of the medium, of:

- (i) at least one first nucleic acid target having a sequence selected from the group constituted of SEQ ID NO: 1-49, complementary sequences thereof, and homologous sequences thereof, or
- (ii) at least one fragment of said first nucleic acid target, wherein said fragment is preferably not constituted of or comprised in a sequence selected from the group constituted of SEQ ID NO: 111-140;

whereby, if said first nucleic acid target or fragment thereof is present in the sample, it is determined that *Ralstonia solanacearum* race 3 biovar 2 is present in the medium.

[0015] The present invention also relates to the use of:

- (i) at least one first nucleic acid target having a sequence selected from the group constituted of SEQ ID NO: 1-49, complementary sequences thereof, and homologous sequences thereof, or
- (ii) at least one fragment of said first target nucleic acid, wherein said fragment is preferably not constituted of or comprised in a sequence selected from the group constituted of SEQ ID NO: 111-140;

for detecting *Ralstonia solanacearum* race 3 biovar 2.

[0016] As intended herein the "medium" can be of natural or synthetic origin. Where the medium is of synthetic origin, it can in particular be a solid or liquid bacterial culture medium, Where the medium is of natural origin, it can notably be water, soil, or a biological tissue, in particular a plant tissue. Preferably, the medium is selected from the group constituted of a potato tissue, a tomato tissue, and a geranium tissue. These plant tissues are particularly prone to infection by *Ralstonia solanacearum* race 3 biovar 2. However, it is particularly preferred that the medium is a potato tissue, since *Ralstonia solanacearum* race 3 biovar 2 is the causative agent of potato brown rot. Preferably, the potato tissue should be the tuber, which constitutes the potato tissue most likely to contain *Ralstonia solanacearum* race 3 biovar 2.

[0017] As intended herein a "sample" relates to a portion of the medium liable to contain nucleic acids. In particular, it is preferred that the sample is obtained from the medium by a nucleic acid extraction. Numerous methods exist, which are well known to one of skill in the art, for extracting nucleic acids from a medium, in particular from a plant tissue.

[0018] As intended herein "nucleic acid" preferably relates to RNA or DNA.

[0019] As intended herein "determination" of the presence or the absence of a nucleic acid in a sample relates to the detection of the full length nucleic acid in itself, but also to fragments of the nucleic acid. The present inventors have shown that genetic material transfers which have occurred in the course of evolution and which have structured the genome of *Ralstonia solanacearum* race 3 biovar 2 concerned genomic patches often involving several genes. These genomic patches correspond to SEQ ID NO: 1-49. Accordingly, the detection of fragments of these genomic patches is generally indicative of the presence of the nucleic acid as a whole. Besides, even if portions of SEQ ID NO: 1-49 should be lacking in a particular race 3 biovar 2 strain of *Ralstonia solanacearum*, it will be clear to one of skill in the art that the detection of fragments of conserved portions of SEQ ID NO: 1-49 is sufficient to unambiguously determine the presence of *Ralstonia solanacearum* race 3 biovar 2.

[0020] Thus, as intended herein a "fragment" should be of a length such that it can be considered by one of skill in the art that it presents essentially no identity with nucleic acids of the same length which can be found in *Ralstonia solanacearum* strains which are not of race 3 biovar 2. Preferably, fragments according to the invention comprise at least 9 nucleotides, more preferably at least 15 nucleotides and most preferably at least 18 nucleotides.

[0021] Exemplary fragments of the above-mentioned genomic patches notably encompass gene and inter-gene sequences SEQ ID NO: 50-110.

[0022] Some short portions of SEQ ID NO: 1-49 are of a known length but of undetermined sequence. It will be apparent

to one of skill in the art that the above-defined fragments of SEQ ID NO: 1-49 should preferably not be constituted of these short portions.

[0023] The inventors have also found that the genomic patches they identified comprised sequence derived from mobile genetic elements. These mobile genetic elements are represented by SEQ ID NO: 111-140. Accordingly, the method and use according to the invention should preferably not determine the presence or absence of these mobile genetic elements or fragments thereof as first target nucleic acids.

[0024] As intended herein "homologous sequences" relate to natural variants of sequences SEQ ID NO: 1-49, which can be found in strains of *Ralstonia solanacearum* race 3 biovar 2. Thus the homologous sequences notably encompass:

- sequences which are derived from sequences SEQ ID NO: 1-49 by insertion, deletion or substitution of at least one nucleotide;
- sequences which are liable to hybridize under stringent conditions to the complementary sequences of SEQ ID NO: 1-49;
- sequences which present at least 95% identity to sequences SEQ ID NO: 1-49;

provided the homologous sequences are specific to *Ralstonia solanacearum* race 3 biovar 2.

[0025] In a particular embodiment of the above-defined method and use, the determination comprises at least one step of hybridization of the nucleic acid target or fragment thereof with a probe or a primer. Preferably, the probe or primer is a fragment of nucleic acid having a sequence selected from the group constituted of SEQ ID NO: 1-49, homologous sequences thereof, and complementary sequences thereof. More preferably, the probe or primer is selected from the group constituted of SEQ ID NO: 141-386.

[0026] In another particular embodiment of the above-defined method and use, the determination comprises at least one step of nucleic acid amplification.

[0027] Preferably, where the determination comprises a step of nucleic acid amplification, the determination is implemented by a method selected from PCR and NASBA. NASBA is notably described in Compton (1991) Nature 350:91-92.

[0028] Also preferably, the determination is implemented by a method selected from Southern blotting, Northern blotting, dot blots, and nucleic acid micro or macro-array hybridization,

[0029] In a particular embodiment of the invention the above-defined method and use also comprise the determination of the presence or the absence in a sample of the medium, of:

- (i) at least one second nucleic acid target having a sequence selected from the group constituted of SEQ ID NO: 111-140, complementary sequences thereof, and homologous sequences thereof, or
- (ii) at least one fragment of said second nucleic acid target.

[0030] Indeed, since the at least one first target sequence is preferably not a mobile genetic elements, the determination of the presence or absence of nucleic acids corresponding to mobile genetic elements can be effected in a further step.

[0031] The present invention also relates to a nucleic acid having a sequence selected from the group constituted of SEQ ID NO: 1-22, SEQ ID NO: 50-110, SEQ ID NO: 111-140, SEQ ID NO: 141-246, and SEQ ID NO: 247-386 and their complementary sequences.

[0032] The present invention also relates to a nucleic acid micro or macro-array comprising a plurality of nucleic acid probes arranged onto a solid support, wherein the nucleic acid probes are fragments of nucleic acids having sequences selected from the group constituted of SEQ ID NO: 1-49, complementary sequences thereof, and homologous sequences thereof, provided that preferably at least one of the nucleic acid probes is not a fragment of a nucleic acid having a sequence selected from the group consisting of SEQ ID NO: 111-140.

[0033] Preferably, in the above-defined nucleic acid micro-array, the nucleic acid probes comprise SEQ ID NO: 247-386.

[0034] The present invention also relates to a kit intended for the detection of *Ralstonia solanacearum* race 3 biovar 2 in a medium, comprising at least:

- two primers suitable to amplify a portion of a nucleic acid having a sequence selected from the group constituted of SEQ ID NO: 1-49 and complementary sequences thereof, provided that said portion is preferably not comprised in a nucleic acid having a sequence selected from the group consisting of SEQ ID NO: 111-140 and complementary sequences thereof;
- optionally one detectable nucleic acid probe suitable to hybridize to said amplified portion.

EXAMPLE

Materials and methods :

5 • *Bacterial strains used in this study*

[0035] **Table 1** provides the list of the 14 race 3 biovar 2 strains and 45 non-race 3 biovar 2 strains used herein together with their geographical origin, host of origin. The 14 race 3 biovar 2 strains corresponded to phylotype IIB, sequevar 1 based on the classification scheme proposed by Fegan & Prior (2005).

10 • *Microarray :*

[0036] A 12X draft of the genome sequence from strain IPO1609 (a race 3 biovar 2 strain accessible from the *Collection Française de Bactéries Phytopathogènes* under accession number CFBP 6926) has been established in collaboration with Genoscope and annotated by the inventors. The deduced amino acid sequence of the genes thus identified was compared with the amino acid sequence of the genes previously identified in strain GMI1000 (Salanoubat *et al.*, 2002). All the genes from IPO1609 that did not have a homolog (less than 40% identity over at least 80% of both the query and subject sequences) will be further referred to as "potential IPO1609-specific genes". For each of these genes a specific representative 70 mer-oligonucleotide was designed using ROSO algorithm (Reymond, 2004). These oligonucleotides were chosen as having no significant homology with any part of the genome of strain GMI1000 and were used to generate a microarray as previously reported for the construction of the GMI1000 microarray (Occhialini *et al.*, 2005). The "IPO1609 partial microarray" thus generated also includes additional oligonucleotides representative of the IPO1609 allelic variants for a limited number of genes conserved but significantly divergent between strains GMI1000 and IPO1609 (mostly including type III-secretion dependant pathogenicity effectors) therefore permitting the distinction of the two allelic forms of a given gene. Finally, a limited number of oligonucleotides representative of particular intergenic regions were also included on the microarray. The sequence of each individual oligonucleotide spotted on the microarray has been deposited at *Agence de Protection des Programmes* (APP) under accession number IDDN.FR.001.300024.000.R.P. 2006.000.10300.

30 • *Genomic DNA extraction, DNA labelling, microarray hybridization, hybridization signal measurement and analysis.*

[0037] These have been performed as previously described (Guidot *et al.*, 2007) except that standard control DNA used for all genome hybridizations experiments which were performed consisted of an equimolar combination of the genomic DNA from three sequenced strains GMI1000, IPO1609 and Molk2 (IPO1609 and Molk2 genomes being unpublished) (Molk2 is accessible from the *Collection Française de Bactéries Phytopathogènes* under accession number CFBP 6925 and from the ATCC under accession number BAA-1115). Analysis was conducted as previously described using ImaGene and GeneShight (BioDiscovery) softwares. A gene was considered as being absent in the tested strain when the base 2 logarithm of the ratio of the normalized hybridization signal of the tested strain over the normalized hybridization signal with the control DNA was lower to -1.

40 • *PCR validation*

[0038] The list of candidate race 3 biovar 2-specific genes deduced from comparative genomic hybridizations (CGH) experiments was checked by PCR amplification. The PCR primers used are given in **Table 2**. The primers were designed to amplify one genomic fragment from each gene. When possible, one of the two primers for each gene was designed inside the oligonucleotide spotted on the microarray. PCR were conducted in 25 µl reaction mixture containing 10 ng DNA from each tested strain, 25 pmol of each primer (UR), 1.5 mM MgCl₂, 200 µM of each four dNTP, 0.5 U of Red Gold Star Taq DNA polymerase (Eurogentec) and the buffer supplied by the manufacturer. PCR amplifications were performed as follows: an initial denaturation step at 96°C for 5 min followed by 30 cycles of 94°C for 15 s., 59°C for 30 s., and 72°C for 30 s., with a final extension step of 72°C for 10 min. Negative (PCR reaction mixture without DNA) and positive (IPO1609 DNA) controls were included in each experiment. The multiplex PCR described by Fegan & Prior (2005) for the *Ralstonia solanacearum* phylotype identification was conducted on each tested DNA as an amplification positive control.

55 • *In silico comparison of strains UW551 with IPO1609, GMI1000 and Molk2 strains*

[0039] The deduced amino acid sequence for each predicted gene from strain UW551 was BlastX compared to the genomic sequence of strain IPO1609 in order to identify the predicted proteins of UW551 that have no counterpart

covering at least 80% of their length. These proteins designated as "potential UW551-specific proteins" were then compared with BlastX to the genomic sequence of strain GMI1000 and Molk2. All the proteins that did not have a counterpart covering at least 80% of the length of the query sequence with at least 40% identity were then individually compared using BlastP with the predicted proteins from strains GMI1000 and Molk2. This was performed in order to eliminate false candidates that could remain in the list due to frameshift in the nucleotide sequence.

Results:

- Analysis of the distribution of "potential IPO1609-specific genes" among a collection of strains representative of the diversity of *Ralstonia solanacearum*.

[0040] Comparative genomic hybridizations (CGH) on the microarray have been performed to compare the lists of genes between 11 race 3 biovar 2 strains and 20 non-race 3 biovar 2 strains (**Table 1**). This analysis identifies a set of 137 oligonucleotides which are present in at least 10 of the 11 race 3 biovar 2 strains and absent from at least 19 of the 20 non-race 3 biovar 2 strains. These oligonucleotides were representative of 79 genes and 38 intergenic regions from the IPO1609 genome. These genes and intergenes therefore were considered as "candidate race 3 biovar 2 specific genomic regions". A large proportion of these regions forms clusters in the IPO1609 genome and sometime a few genes mapping within these clusters were not found in the list of thus defined "candidate race 3 biovar 2 specific regions". Based on

- i) the known mosaic structure of *Ralstonia solanacearum* genome that suggests that sets of genes could have been acquired through horizontal gene transfers or lost through deletions (Salanoubat et al., 2002 ; Guidot et al., 2007),
- ii) on the consideration that some genes might be missing from this list due to the fact that hybridization data are missing in a limited number of cases, and
- iii) taking into account the possibility that certain genes that score next to the cutoff value could have been miss-categorized,

the inventors included into the list of the "race 3 biovar 2 specific regions" all the sets of 1 or 2 contiguous genes that were not originally detected as being race 3 biovar 2 -specific but that are located within a race 3 biovar 2 -specific gene cluster. Based on these criteria 34 additional genes were included into the list of race 3 biovar 2 -specific genes. These additional genes are identified with a star (*) in **Table 3**.

[0041] When compared using BlastP with the sequence of the predicted proteins from strains GMI1000 and Molk2, 5 of these genes were found to have a counterpart in at least one of these strains and were therefore eliminated from the final list of race 3 biovar 2 -specific genes presented in **Table 3**. This final list includes a total of 151 genes or intergenic regions organized in 18 clusters and 15 additional individual genes or intergenic regions.

- Validation of candidate race 3 biovar 2-specific genomic regions.

[0042] The list of candidate race 3 biovar 2-specific genomic regions given in **Table 3** has been established based on the analysis of a limited number of strains. Therefore the inventors validated this list on a larger collection of strains representative of the diversity found in *Ralstonia solanacearum*.

[0043] A total of eight race 3 biovar 2-strains (among-which six were also used for CGH experiments) and 32 non-race 3 biovar 2 strains (seven of which were also used for CGH experiments) were used for this validation (**Table 1**). This validation was conducted by PCR amplification of one genomic fragment from each candidate race 3 biovar 2-specific region. All tested DNA could be amplified using the multiplex PCR for the *Ralstonia solanacearum* phylotype identification as described by Fegan and Prior (2005) therefore confirming that the strains tested actually correspond to *Ralstonia solanacearum* isolates.

[0044] Each genomic region that gave a positive amplification from non-race 3 biovar 2 strains or a negative amplification from race 3 biovar 2 strains was excluded from the list of "race 3 biovar 2 specific genomic regions". The results validated the specificity of eleven gene clusters and eleven individual genes or intergenic regions (**Table 4**). Among these genes, 27 were predicted to be parts of mobile genetic elements (bacteriophage or insertion sequences).

- Identification of additional candidate race 3 biovar 2-specific genes in strain UW551

[0045] A genomic draft for the race 3 biovar 2 virulent isolate UW551 is publicly available (Gabriel et al. 2006). Because strain IPO1609 probably harbours a genome deletion that significantly impairs its virulence compared to UW551, the inventors decided to compare the genome sequences for these two strains. In doing this 328 predicted genes from UW551 that had no counterpart in IPO1609 were identified. Based on the same criteria, *in silico* comparison of these

328 genes with the genome sequence of strain Molk2 and GMI1000 reduced this set to 94 UW551 specific genes. Comparison of these 94 predicted proteins using BlastP with the proteins of GMI1000 and Molk2 eliminated 67 proteins that were not previously detected as having a counterpart in GMI1000 or Molk2 due to the presence of a potential frameshift mutation in the corresponding genes, therefore leading to a final list of the 27 UW551 specific genes shown in **Table 5**.

List of tables

[0046]

Table 1: List of *Ralstonia solanacearum* strains used in this study (ND not determined, NO no object)

Table 2: List of the oligonucleotides used for PCR amplification of candidate race 3 biovar 2 specific genes. (a) Sequences in bold correspond to part of the oligonucleotide spotted on the microarray

Table 3: List of candidate race 3 biovar 2-specific genomic regions.*Added genes correspond to the genes that have been included into the list based on their location within clusters of race 3 biovar 2 specific gene clusters.

Table 4: Validated race 3 biovar 2 -specific genomic regions together with sequences of the PCR primers used for gene amplification. Black boxes highlight genes from mobile genetic elements (bacteriophage or insertion sequences).

Table 5: List of additional candidate race 3 biovar 2-specific genes identified from strain UW551.

Table 1

Strain ID	Host	Origin	Race	Biovar	CGH on microarray	PCR verification
IPO1609	Potato	Netherlands	3	2	Yes	control +
JT516	Potato	Reunion Is.	3	2	Yes	Yes
CMR34	Tomato	Cameroon	3	2	Yes	Yes
RE	Potato	Uruguay	3	2	Yes	Yes
AP31 H	Potato	Uruguay	3	2	Yes	
AP42H	Potato	Uruguay	3	2	Yes	Yes
TB1H	Potato	Uruguay	3	2	Yes	
TB2H	Potato	Uruguay	3	2	Yes	
TC1H	Potato	Uruguay	3	2	Yes	Yes
TB10	Potato	Uruguay	3	2	Yes	
ETAC	Potato	Uruguay	3	2	Yes	
RM	Potato	Uruguay	3	2	Yes	Yes
PSS525	Potato	Taiwan	3	2		Yes
CMR24	Potato	Cameroon	3	2		Yes
CIP10	Potato	Peru	3	2T		Yes
NCPB3987	Potato	Brazil	3	2T		Yes
Molk2	Banana	Philippines	2	1	Yes	
CIP418	Peanut	Indonesia	2	1	Yes	
UW9	Heliconia	Costa Rica	2	1		Yes
CFBP1183	Heliconia	Costa Rica	2	1		Yes
UW163	Plantain	Peru	2	1	Yes	
Ant75	Heliconia	Martinique	NO	1	Yes	
Ant80	Anthurium	Martinique	NO	1	Yes	
Ant307	Anthurium	Martinique	NO	1	Yes	
JY200	Anthurium	Martinique	NO	1	Yes	
JY201	Anthurium	Martinique	NO	1	Yes	
Ant1121	Anthurium	Martinique	NO	1	Yes	Yes

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(continued)

	Strain ID	Host	Origin	Race	Biovar	CGH on microarray	PCR verification
5	CFBP6797	Solanum	Martinique	NO	1		Yes
	CFBP7014	Anthurium	Trinidad	NO	1		Yes
	TOM=T1	Tomato	Uruguay	2	1	Yes	Yes
	B34	Banana	Brazil	2	1	Yes	Yes
	A3909	Heliconia	Hawai	2	1	Yes	Yes
10	CIP239	Potato	Brazil	1	1		Yes
	CIP301	Potato	Peru	1	1		Yes
	CFBP2957	Tomato	Martinique	1	1		Yes
	CMR39	Tomato	Cameroon	1	1		Yes
	ICMP7963	Potato	Kenya	1	1		Yes
15	CFBP6942	Solanum	Cameroon	NO	2T	Yes	Yes
	CFBP6941	Tomato	Cameroon	NO	2T	Yes	
	CMR43	Potato	Cameroon	NO	2T		Yes
	CIP358	Potato	Cameroon	NO	2T		Yes
20	CFBP3059	Eggplant	Burkina Faso	NO	1	Yes	Yes
	CMR66	Solanum	Cameroon	NO	2T		Yes
	JT525	Pelargonium	Reunion Is.	NO	1		Yes
	JT528	Potato	Reunion Is.	NO	1		Yes
25	J25	Potato	Kenya	NO	2T		Yes
	NCPB332	Potato	Zimbabwe	NO	1		Yes
	GMI1000	Tomato	Guyana	1	3	Yes	
	CMR134	Solanum	Cameroon	1	3	Yes	
	CIP365	Potato	Philippines	1	3		Yes
30	R288	Morus alba	China	1	5		Yes
	PSS358	Tomato	Taiwan	1	3	Yes	
	PSS190	Tomato	Taiwan	1	3	Yes	
	PSS219	Tomato	Taiwan	1	3		Yes
35	ACH732	Tomato	Australia	NO	2		Yes
	Psi07	Tomato	Indonesia	NO	2T		Yes
	Psi36	Tomato	Indonesia	NO	2T		Yes
	MAFF301558	Potato	Japan	NO	2T		Yes
40	R. syzygii	Clove	Indonesia	NO	NO	Yes	Yes
	R28						

Table 2

	Gene name	Primer name	Primer sequence (a)	Product size (bp)	SEQ ID NO : 1
45	IPO_00030	IPO_00030_L	ACTTGGAGAGATTTACGGAGGAG	200	387
		IPO_00030_R	AAGCAAACGAGATAAGGGAGAAC		388
	IPO_00031	IPO_00031_L	AAACACAATTCACCTTCCTGATG	185	389
50		IPO_00031_R	GGCCACTAGACTTTCCAGTGAT		390
	IPO_00034	IPO_00034_L	AGCTTACCTGCTGTTGACATCT	818	391
		IPO_00034_R	GTTCTTCGTGATAGCGGAGACT		392
	IPO_00043	IPO_00043_L	CTGAATTCGAAAAGGATAGAGCA	206	141
		IPO_00043_R	CTCGACAACTCTTGCAACTGAC		142
55	IPO_00044	IPO_00044_L	CTATGCAGAAGCGTTGCTTGTT	191	143
		IPO_00044_R	CTTTAGCGAGCACAAAGATTGAGT		144
	IPO_00045	IPO_00045_L	GAGATCGTTGAAACATCAAGAC	165	145

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(continued)

	Gene name	Primer name	Primer sequence (a)	Product size (bp)	SEQ ID NO : 1
		IPO_00045_R	GTGAACCACTATTGCCGGTATC		146
5	IPO_00233	IPO_00233_L	AGAACTGCCAAGTTCGACTACCT	207	147
		IPO_00233_R	CATTCCAACGTTCAAGTGGTTAT		148
	IPO_00234	IPO_00234_L	GCAGAAAAGATATCCCCTGCAC	214	149
		IPO_00234_R	TTCGAGTACAAATGTAGGCTTCC		150
10	IPO_00875	IPO_00875_L	GATCAGATGGAGCAAAGAACAAC	221	151
		IPO_00875_R	TATTGAAACTCTTCACGGGTCAT		152
	IPO_00876	IPO_00876_L	TTTCGACCAAGAAAAGCATAGAG	238	153
		IPO_00876_R	ATTTCTGTGCCCACTACGAAC		154
	IPO_00877	IPO_00877_L	TGGTGTCTAACTGTGGAAGGTT	163	155
15		IPO_00877_R	TACCGCCAGTCATATCAGTTCTT		156
	IPO_00878	IPO_00878_L	ATTAGACTGATCAAGGCATGGAA	152	157
		IPO_00878_R	CCTTCATTATTGAGACGGTCAAG		158
	IPO_00879	IPO_00879_L	ATGTTTGTGCTACTGGTCAGTCC	223	159
		IPO_00879_R	CCTTCACTTGCAGATAATGGAAC		160
20	IPO_00881	IPO_00881_L	AAAGAAGCTCAAGGAGATCAAGG	201	161
		IPO_00881_R	AACAGCAGGTTGTGATACTGCAT		162
	IPO_01030	IPO_01030_L	TATGAATGGGTTGATAGCGTTCT	165	393
		IPO_01030_R	CCATATCCACCGATAAACAACAT		394
25	IPO_01058	IPO_01058_L	CGAGCTCATCGTTATCGACAT	140	163
		IPO_01058_R	AAGCTCTTGGACTAGGACGATCT		164
	IPO_01131	IPO_01131_L	CACGATATGACCACGATCAACTA	195	395
		IPO_01131_R	GTAGACACGAATCACGTCTCCAT		396
	IPO_01132	IPO_01132_L	ACATCAACGACCCTTACTGTCC	147	397
30		IPO_01132_R	GACCATAGTCATCGCTGCTTAAC		398
	IPO_01137	IPO_01137-1_L	CTATCCCGCAGAAGGTATTCAAC	186	399
		IPO_01137-1_R	AGTCATAGGCGTCTCGGTACTT		400
		IPO_01137-2_L	ACACTCTGTTACCAAGTACGG	217	401
35		IPO_01137-2_R	CTTTGAAACTGGAGGAACAGCTT		402
	IPO_01259	IPO_01259_L	TGAAATGCTCAAAGACAAACAGA	235	165
		IPO_01259_R	ATCGTACAGGTCATTGCCAAAT		166
	IPO_01260	IPO_01260_L	TACAACCTGAAGAGGATCTCGAA	225	167
		IPO_01260_R	AAAGCCGGTCATAGAGGACATAG		168
40	IPO_01311	IPO_01311_L	CAACCAGACCATCTACAAGATCC	165	169
		IPO_01311_R	GCTTCATACTCAAATCGAACACC		170
	IPO_01312	IPO_01312_L	AACTCCAACCTGCTTGAAGTTC	208	171
		IPO_01312_R	GGATGAACCTCGTTCGATTGAG		172
45	IPO_01314	IPO_01314_L	GAAGCTCGGTGATATCGAAAC	287	173
		IPO_01314_R	GGTGATCGCTGTCGATAATTT		174
	IPO_01362	IPO_01362_L	AATTGGGTATACGTGATCTGTGG	280	243
		IPO_01362_R	TCGGGTAAGACGAAGCTGACTA		244
	IPO_02090	IPO_02090_L	CAATAGAAATTGCCGAGGTGATA	171	239
50		IPO_02090_R	CCTTGATAAGGATGTTCAACGAC		240
	IPO_02092	IPO_02092_L	AACACTCAAAAGCTGACCATCAT	151	403
		IPO_02092_R	CAACCTTGATCTGTTCCGAGAC		404
	IPO_02095	IPO_02095_L	GTTGCAATGCTGGTTTCCAAG	157	405
		IPO_02095_R	GTCATGGACGAGAAATCGATAC		406
55	IPO_02097	IPO_02097_L	CTCAGAGGATCTGTTTCATCGACT	160	407
		IPO_02097_R	GTTGAAGACGCCGAAGAAAAA		408
	IPO_02098	IPO_02098_L	ACGAGATTCTGAAGCTGAGGT	192	409

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(continued)

	Gene name	Primer name	Primer sequence (a)	Product size (bp)	SEQ ID NO : 1
		IPO_02098_R	CTTGCGAACCTGACACATGA		410
5	IPO_02102	IPO_02102_L	GTATCAGAAAGGCCAGCTACACA	207	175
		IPO_02102_R	CTGATTGCCAATATTCGATTCTC		176
	IPO_02140	IPO_02140_L	AAGGGCAATGGCTTTTTCTGT	153	177
		IPO_02140_R	GAGACTGATAAATCAGCGTTTCC		178
10	IPO_02141	IPO_02141_L	GCTTTCTACGTCGCCTCAGTAT	225	179
		IPO_02141_R	GTAACGGTCTGATTCTTGAGGTTT		180
	IPO_02142	IPO_02142_L	CCACTACCCCTGTTTCCATTC	245	181
		IPO_02142_R	AAACCTGTAGTCGTCGTCCTTG		182
	IPO_02143	IPO_02143_L	AGACGTTGGACAACATCAACC	189	183
15		IPO_02143_R	AGTTCTGTTTCGTCGTCGTAAT		184
	IPO_02144	IPO_02144_L	CCAGTATTCGAAGACGCTATCC	189	185
		IPO_02144_R	AACGGATACAGCAGCAGGTTT		186
	IPO_02145	IPO_02145_L	ATACCCAGCGCGTATTCCAA	164	187
		IPO_02145_R	GTTGAGGCAACACCAGACAG		188
20	IPO_02146	IPO_02146_L	CTTGAGAAAGCTTTTGGTGAAGA	166	189
		IPO_02146_R	CTTTGAGACCTTCCCAGGCTAA		190
	IPO_02147	IPO_02147_L	TAAGAGCAGGCTATGGACAACAT	209	191
		IPO_02147_R	AATACCAGCACACAGAAGGTCAG		192
25	IPO_02148	IPO_02148_L	CTGATTTCCATGTACCTGCATC	235	193
		IPO_02148_R	ACATCAGCACGTTCTTGTAGAGC		194
	IPO_02149	IPO_02149_L	GACGATGAGTTGCTGAAGTACC	237	195
		IPO_02149_R	TGAAGGTAATGGTCACAGCTTTT		196
	IPO_02150	IPO_02150_L	ATCCGGTTCCTTCTGATGATCT	124	197
30		IPO_02150_R	CTTCAACTTCACGTGTTCAATCA		198
	IPO_02151	IPO_02151_L	GCTAGTCCACACGACAAAATCAT	163	199
		IPO_02151_R	GTGACTAGCTTGGCGATCTTCT		200
	IPO_02152	IPO_02152_L	AGGTGAAGTGCTCGAAATCCT	284	201
35		IPO_02152_R	CTTCTTCCTTCTCGATGCCTTC		202
	IPO_02153	IPO_02153_L	TTATGTGGCTTTCTCTGGCAATA	228	203
		IPO_02153_R	ACAAACGTCCAGTCGTCAATC		204
	IPO_02154	IPO_02154_L	CATTATCTCTGGTCTTGGCTTG	173	205
		IPO_02154_R	ACAGCCAAACTGACAAGATCG		206
40	IPO_02155	IPO_02155_L	GCATGAAAATGTCTACGTTCCCTC	491	207
		IPO_02155_R	ATGGTGATAGTGCGAATGAC		208
	IPO_02156	IPO_02156_L	CAGACATGTTCTGCGAAGGAT	283	209
		IPO_02156_R	GAGGAACGTAGACATTTTCATGC		210
45	IPO_02157	IPO_02157_L	TGTAATGACCAAGCAAGAACTCA	112	211
		IPO_02157_R	CACGGTGTCAAGAATGGTTTC		212
	IPO_02158	IPO_02158_L	ATTTCAAACGCCAAGCCTTTAAC	165	213
		IPO_02158_R	GTTTCATCGAAAGCGATGTTCTC		214
	IPO_02159	IPO_02159_L	GCCTATTTGGACCGAAGAAGAC	394	215
50		IPO_02159_R	ACTTCTTGAGGCATCTCGGTTT		216
	IPO_02160	IPO_02160_L	GCAGCATTGATGACAAGTTCC	339	217
		IPO_02160_R	AGTATTGGTAAAGGCGTTGCAC		218
	IPO_02162	IPO_02162_L	AAGGCTAAGGGGGAGTAAGTCAT	548	411
		IPO_02162_R	AGGTAGTTGCGTACTTGGTCGTA		412
55	IPO_02163	IPO_02163_L	AGCAACCGAAGATATCGACCT	284	219
		IPO_02163_R	GCCCAAGCGAAATCAACTCTT		220
	IPO_02165	IPO_02165_L	CTTCCCAGTCAATACATTCCAG	220	221

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(continued)

	Gene name	Primer name	Primer sequence (a)	Product size (bp)	SEQ ID NO : 1
		IPO_02165_R	CGTTATCACATAAGCCACATCAA		222
5	IPO_02166	IPO_02166_L	AAACAACACGCTGTTGAGCAT	195	223
		IPO_02166_R	CTTCTTTGCGCACCAATAATC		224
	IPO_02923	IPO_02923_L	GCGCAAATTAACACTACAAAGG	225	413
		IPO_02923_R	ATCCCCAATCTCCTTTATCACTC		414
10	IPO_02924	IPO_02924_L	GAAGGTAAATCCAAAGGAAATGG	232	415
		IPO_02924_R	AATTGACTTCGGCTCGTATTCTT		416
	IPO_02927	IPO_02927_L	AATTCCATGAGTGAGTGATTTTT	245	417
		IPO_02927_R	AGATCATGAGCCTCAGCATTATTA		418
	IPO_03123	IPO_03123_L	GAACGGAGCCATAGTGATGAAG	946	419
15		IPO_03123_R	AGAGTCGCTAACACAAGTCATCC		420
	IPO_03132	IPO_03132_L	AGGGAATCAAATCGCTCATCT	226	245
		IPO_03132_R	AGAAGAAGCCCATGATGACAGAG		246
	IPO_03302	IPO_03302_L	CGTGTATATCGGCAGTCAAGAAG	227	225
		IPO_03302_R	CTAAGAAATGAAAGGTGGGGTTC		226
20	IPO_03306	IPO_03306_L	GGATGACTTGTGTTAGCGACTCT	171	227
		IPO_03306_R	GAATACGATCCTCCACAATCAAA		228
	IPO_03560	IPO_03560_L	CACGATCATATATGGGTCCAGTT	173	421
		IPO_03560_R	GGTTCTTTTTGATCGTAGCCTTT		422
25	IPO_03656	IPO_03656_L	TCTGTTCCGAGTATCACCTTGTT	156	423
		IPO_03656_R	CATAGTATTCGCAGTCCAGATCC		424
	IPO_03659	IPO_03659_L	GGATGTGGGGAGGTTTATTAGTC	192	425
		IPO_03659_R	TTCATCAACTCCTCAGGAATCAG		426
	IPO_03742	IPO_03742_L	AAGATATGTGCCAGCTACCACTG	206	427
30		IPO_03742_R	AGCATATACAGTCCCTCGTATGC		428
	IPO_03816	IPO_03816_L	ATTTCAACGACCTGCATCAGA	272	429
		IPO_03816_R	CCACACCAGGTTCTTCTTGTTT		430
	IPO_04000	IPO_04000_L	GTAAGGTCTGCAAGGACATCATC	179	431
35		IPO_04000_R	GTGGCTGCGATAGAAGTTGTAGT		432
	IPO_04001	IPO_04001_L	GACCATCTTTCAGTGGTGGA	110	433
		IPO_04001_R	TAATGCCCTAAACTTTCTGATG		434
	IPO_04002	IPO_04002_L	TGTTTGATCTGAGCAAGTTGTG	250	435
		IPO_04002_R	AGAAACTCATCCGCAAGGTC		436
40	IPO_04003	IPO_04003_L	GTAAGGAGAAGTGATGTCGGAAC	283	437
		IPO_04003_R	GTCCTTGTTCTTGAAGTGGTACG		438
	IPO_04004	IPO_04004_L	CTTGACGTCTGACAACCAAGTAG	342	241
		IPO_04004_R	ATAAGATAAACAGGTCGGCCTTC		242
45	IPO_04067	IPO_04067_L	CATGCCAAGGAACACATCAAG	203	439
		IPO_04067_R	AAGTATTTGTTGCCGTGGTACTC		440
	IPO_04353	IPO_04353_L	AGGTACTCGGCTATCACAATCAC	202	441
		IPO_04353_R	ATGCATTCTCCATGTATTCCATC		442
	IPO_04521	IPO_04521_L	TGAAGCACTGTCTATCAACCAGA	222	229
50		IPO_04521_R	TTTGTCTAGTCACAGCACTGAA		230
	IPO_04523	IPO_04523_L	GACTTCGGCTATCTGGAGAAAAT	217	231
		IPO_04523_R	TCTTAGCAGGTTTAGGCTGAGTG		232
	IPO_04524	IPO_04524_L	CATCTTCAAGGATGACTCTCTGG	235	233
		IPO_04524_R	GAAGAAGTGACCAGGCTGAATTT		234
55	IPO_04525	IPO_04525_L	ACTCAGTGACGAAGAGGTTGAAG	250	235
		IPO_04525_R	ACGAGTAGCTTCAATGGTGCTT		236
	IPO_04526	IPO_04526_L	ACACTATGCCTGCTGACTTGAA	169	237

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(continued)

Gene name	Primer name	Primer sequence (a)	Product size (bp)	SEQ ID NO : 1
	IPO_04526_R	AGGTGACTTCAACAATGTTAGGC		238
5 IPO_04530	IPO_04530_L	GAGACTCGGTTCAACAAGAAAAA	207	443
	IPO_04530_R	CTTAGACGCACTAGCGAAATACG		444
a) sequences in bold correspond to part of the oligonucleotide spotted on the microarray				

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Table 3

Race 3 biovar 2 specific genes clusters

	Oligo ID	Gene ID	SEQ ID NO :	Oligonucleotide sequence
1	IPO1609_0648	IPO_00030	247	GGCTCGAACAACTTGGAGAGATTTACGGAGGAGACGTGTCATTGCTCATGCCGAATTCACGCTACGCCGC
1	IPO1609_0647	IPO_00031	248	CCGGAGGAACCGCTGCGATGGACGCCATCACTGGAAAGTCTAGTGGCCGAACCGCGAAAGCGCGCGCATG
2	IPO1609_0644	IPO_00043	249	GTGACGATACCGCTGTTGCGTCTCTGTCGTTTGTGTCCAAGAGGGCTTCAAACCAGATACGTTCTCCGC
2	IPO1609_0643	IPO_00044	250	GCGAACAAATGTCACCGTCGCCTATGCAGAAGCGTTGCTTGTGTCACGCCTGCCTCTCGTCTTGTGATG
2	IPO1609_0641	IPO_00045	251	CAAGGTCTTTGACGAGATCGTTGGAACATCAAGACCATTGGGCTAAAGAAGCCAATCACCGTCACGCCG
2	IPO1609_0642	IPO_00045	252	GACGAGAATTTCTCAACCTTCTGCGAGCGGAATCTTTGGCGACACTTCCCAAATATCTGGCTGAGCGTG
2	IPO1609_0640	IPO_00046	253	CGCAACCGTGAGGGAGGTGCTCACCAACGAGAAATACATCGGCAACAACATCTATAACCGTGTGTCGTAC
2	IPO1609_0639	IPO_00046/00047	254	GCGCCTGGTTGTCGCGATAGTCGTCATCAGAGCGCAAGTGCTGCTGTTGCCACCGTCGGCGACGTTGGCG
3	IPO1609_0625	IPO_00232/00233	255	GGTGCAGCGTTCTTCTCGGCTCCAGATAAGGCGGTCTGCTACTGTCGTTGATATCTCACAGACGACC
3	IPO1609_0626	IPO_00232/00233	256	GGCTGTGAGTATGTTCTGAGGGTAGCCGCGCATCATCGTCCCGAACCGTTTCGTCAACTGGTAAGTTGG
3	IPO1609_0624	IPO_00233	257	CCTGGTTGTGCTTGATAACCATCTGAACGTTGGAATGCAAGTGCTGGAAGACTCGCTATGCAACTGGCAG
3	IPO1609_0623	IPO_00234	258	CGGAAGTAGGCACATACAAGCACGCCGTCGGAAGCCTACATTTGTAAGTGAAGAACACGGACCGGCGAC
4	IPO1609_0267	IPO_00874/00875	259	GCAGGATAAGAGCCGTGAGGTTCTGTTTCATCTGCGTAATGCACCAACAAAGCTCATGAAGGAACTCGGC
4	IPO1609_0266	IPO_00875	260	GAGCGGTTCTTGAAGCTATGCTGGAGCCATCATCACTGATCCTGCTACGGCGATTGTGGAGCTTGTGCG
4	IPO1609_0265	IPO_00875/00876	261	GTCTTCATCAACCACATTGAAGTTCACGACAAGCCAGCGGCAGACAACATCAGGAACATTGTGTTGCGCG
4	IPO1609_0272	IPO_00876	262	CTGCCTATCATCCTGGATAGAGGGCGAAGCCGAACCTTGGAGCGCACAGTCAAGGAAGGCTTTGAACATG
4	IPO1609_0271	IPO_00877	263	CGGCTACAGGCAGTCTCAAAGAGACAGGTCTGAAGAAGACTATGCCATCTATTGGGTATGTGGCTGGTGC
4	IPO1609_0270	IPO_00878	264	CGTTGAACATGTGGATGGTAAGCACGGAGGTAAAGTCAGGCAGTTGATTAGACTGATCAAGGCATGGAAG
4	IPO1609_0269	IPO_00878/00879	265	CGCTGGTGCTGTGGTGGGCTCCCAAGTGAGTAAGAGCAGTGAAGTGAAGAATGTCACTGACTTGCAAGTG
4	IPO1609_0268	IPO_00879	266	CCAGCAATCCCACTAATGTTTGTGCTACTGGTCAGTCCTACGGCTTCAACTTCGCCAACAATCAATACAC
4		IPO_00880	*	no representative oligonucleotide on the micro array
4	IPO1609_0659	IPO_00881	267	GGAGGTTTGGAGGCTCAGATCGGTGGTTTGTCCATGCAGTATCACAACCTGCTGTTGCTGCTGGCTGGTG
4		IPO_00882	*	no representative oligonucleotide on the micro array
4		IPO_00883	*	no representative oligonucleotide on the micro array
4	IPO1609_0151	IPO_00883/00884	268	GCTGTAGCACCGACTCTGACCGCCAATGACGAAGTTCTATTGAACTTCATTAGTGAATACGGTGATGGTG
5	IPO160_0141	IPO_01057/01058	269	CGTCTTGTGCCTATAGCAATGAAATGCAGAAGGCTTGGCGGACAATATCAACTATGCCAGCTCGTGGTG
5	IPO160_0135	IPO_01058	270	ATCATGGCTGAGATCCAGCGTATTGCCGCAACCGAGCTCATCGTTATCGACATGGGCGACAGGCTTGGCA

	Oligo ID	Gene ID	SEQ ID NO:	Oligonucleotide sequence
19	IPO1609_0147	IPO_01025/01026	372	AGGCTATACGGACAGCACAGCCGCAAATTATGCCTGCCGACTGGGAGGTGAAAGAGGTTGGTGGCACGTT
20	IPO1609_0146	IPO_01030	373	ACATGTTGTTTATCGGTGGATATGGCTGGGATGGCTCGAATCTGTATATTGAAATTAATACAGGAAAGGT
21	IPO1609_0185	IPO_01362	374	CAATTGGGTATACGTGATCTGTGGGTGCCTGATGGGATTGCGGTTGCGCTGGATTGCCATGCTCTGGCTC
22	IPO1609_0233	IPO_02072/02073	375	GCGGTAGCCGACTGGCACACGTATTTGGACGGCGTTGGCCGGGAAGATTTCCCTGACCAACCAGACGATC
22	IPO1609_0234	IPO_02072/02073	376	CAGCCATTCTCTTCCAACCTTATTTCTACTGGATGGATCGCTATTACGTGTTTGCGCCGATGTGGAGCTC
23	IPO1609_0111	IPO_02448/02449	377	CTTGAACCAAGCATAGAGCGCGTCGCATATGCCAGGCCGTGTTCAAGCGTGACCTGATCATCGGTAAGC
24	IPO1609_0563	IPO_03105/03106	378	GCCTATCGTCAACATAGGTTTCCTATTGTTGCTCGAAAGTCAGCGTGGAAATATGCTCAATTTGAACGTGCCG
25	IPO1609_0508	IPO_03123	379	CGCGTGACCAAACGCCAGCGCTTCCTGGCAGAGATGGAGAAGGTCTTG
26	IPO1609_0557	IPO_03132	380	CAAACATTGCCTTCACGGAAGATGAGCAGCATCTGTACAACCTGACGCTTGATGAGCTGGGCGACGAGTC
27	IPO1609_0420	IPO_03433	381	CAATAGCCTTGCCAAGCACTGCAACATGAAGCGCGTCACGGATTTCAACGACCTGCATCAGAAGCAACTG
28	IPO1609_0313	IPO_04287	382	GCCGGTTGCTGACGGAGATCGAGAGGCTGGCGGATGACTTTAGGGAACCGTGATCAATGAAGTCGGCGA
29	PT04834A	IPO_04353	383	CACAACCCTGCGCATTTCCGCGCCGAACTCTTGATGGAATACATGGAGAATGCATCCCCCTCAAAAAACG

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384 GATATCGCGGACATAAGATGCTTCGTCGCCCTCGATGGCTCGTCGTATTTTCGCTAGTGCCTCTAAGGCTG
385 GTCCAGACCTGTCTGTCACACTTGCCTACCAGCAGTCTTACAACGACTACGGATCGAATGTCGGCATCGG
386 GGTCGCCACATTACATTGATGGCCCACTCGACGTATTGAACAAAGTTCCAGGCATTGATCATTGGTGGG

Table 4

Clusters	SEQ ID NO:	Gene ID	SEQ ID NO:	L_PCR primers	SEQ ID NO:	R_PCR Primer	SEQ ID NO:
2	1	IPO_00043	50	CTGAATTCGAAAAGGATAGAGCA	141	CTCGACAAACTCTTGCAACTGAC	142
2		IPO_00044	51	CTATGCAGAAGCGTTGCTTGT	143	CTTTAGCGAGCACAAGATTGAGT	144
2		IPO_00045	52	GAGATCGTTGGAAACATCAAGAC	145	GTGAACCACTATTGCCGGTATC	146
2		IPO_00046	53				
2		IPO_00046/00047	54				
3	2	IPO_00232/00233	55				
3		IPO_00233	56	AGAACTGCCAAGTTCGACTACCT	147	CATTCCAACGTTTCAGATGGTTAT	148
3		IPO_00234	57	GCAGAAAAGATATCCCCTGCAC	149	TTCGAGTACAAATGTAGGCTTCC	150
4	3	IPO_00874/00875	58				
4		IPO_00875	59	GATCAGATGGAGCAAAGAACACT	151	TATTGAAACTCTTCACGGGTCAT	152
4		IPO_00875/00876	60				
4		IPO_00876	61	TTTCGACCAAGAAAAGCATAGAG	153	ATTTCTGTGCCCACTACGAACTA	154
4		IPO_00877	62	TGGTGTCTAACTGTGGAAGGTT	155	TACCGCCAGTCATATCAGTTCTT	156
4		IPO_00878	63	ATTAGACTGATCAAGGCATGGAA	157	CCTTCATTATTGAGACGGTCAAG	158
4		IPO_00879	64	ATGTTTGTGCTACTGGTCAGTCC	159	CCTTCACTTGCAGATAATGGAAC	160
4		IPO_00880	65				
4		IPO_00881	111	AAAGAAGCTCAAGGAGATCAAGG	161	AACAGCAGGTTGTGATACTGCAT	162
4		IPO_00882	112				
4		IPO_00883	113				
4		IPO_00883/00884	66				
5	4	IPO_01057/01058	67				
5		IPO_01058	68	CGAGCTCATCGTTATCGACAT	163	AAGCTCTTGGACTAGGACGATCT	164
7	5	IPO_01258/01259	69				
7		IPO_01259	70	TGAAATGCTCAAAGACAAACAGA	165	ATCGTACAGGTCATTGCCAAAT	166
7		IPO_01260	114	TACAACCTGAAGAGGATCTCGAA	167	AAAGCCGGTCATAGAGGACATAG	168
8	6	IPO_01311	71	CAACCAGACCATCTACAAGATCC	169	GCTTCATACTCAAATCGAACACC	170
8		IPO_01312	72	AACTCCAACCTTGCTTGACTGTTC	171	GGATGAACTTCGTTTCGATTGAG	172

Individual Genes	SEQ ID NO:	Gene ID	SEQ ID NO:	L_PCR primers	SEQ ID NO:	R_PCR Primer	SEQ ID NO:
10A	12	IPO_02090	104	CAATAGAAATTGCCGAGGTGATA	239	CCTTGATAAGGATGTTCAACGAC	240
17	13	IPO_04004	105	CTTGACGTCTGACAACCAAGTAG	241	ATAAGATAAACAGGTCGGCCTTC	242
21	14	IPO_01362	106	AATTGGGTATACGTGATCTGTGG	243	TCGGGTAAGACGAAGCTGACTA	244
22	15	IPO_02072/02073	-				
25	16	IPO_03123	140				
26	17	IPO_03132	107	AGGGAATCAAATCGTCTATCT	245	AGAAGAAGCCCATGATGACAGAG	246
28	18	IPO_04287	108				
29	19	IPO_04504/04505	-				
30	20	IPO_04530	109				
31	21	IPO_04923	110				
32	22	IPO_02934/02935	-				

Table 5

	Gene ID	GenBank Accession number	SEQ ID NO:
5	RRSL_00004	NZ_AAKL01000174	23
	RRSL_00093	NZ_AAKL01000099	24
	RRSL_00419	NZ_AAKL01000074	25
	RRSL_00442	NZ_AAKL01000066	26
	RRSL_00500	NZ_AAKL01000026	27
10	RRSL_00600	NZ_AAKLO1000073	28
	RRSL_00696	NZ_AAKL01000059	29
	RRSL_00772	NZ_AAKL01000058	30
	RRSL_01930	NZ_AAKL01000033	31
15	RRSL_01998	NZ_AAKL01000044	32
	RRSL_02069	NZ_AAKL01000022	33
	RRSL_02232	NZ_AAKL01000025	34
	RRSL_02410	NZ_AAKL01000024	35
	RRSL_02412	NZ_AAKL01000024	36
20	RRSL_02430	NZ_AAKL01000024	37
	RRSL_03158	NZ_AAKL01000027	38
	RRSL_03244	NZ_AAKL01000017	39
	RRSL_03346	NZ_AAKL01000012	40
25	RRSL_03351	NZ_AAKL01000012	41
	RRSL_03359	NZ_AAKL01000012	42
	RRSL_03472	NZ_AAKL01000008	43
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	RRSL_03795	NZ_AAKL01000009	45
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<213> *Ralstonia solanacearum*

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 <213> *Ralstonia solanacearum*

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<213> *Ralstonia solanacearum*

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<213> Ralstonia solanacearum

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<210> 88

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<212> DNA

<213> Ralstonia solanacearum

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 45 accggcaaat ccaacaccta cgaatggctg agccagttcc cggccttcgg ggaatgggtc 180
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 50 accatcgccg agtcggccgg ccagtcggcc accgatctga agaacgatct ggtgtttcaa 360
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 cattcggtgt acgagaacga agacggcacc ggtgccgtca ccaacgtgag caacatgcag 480
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ctgctctgcg gcccgacaa catggccgat gccgaagcgc tgctgaaggc cgcgcagaa 840
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	cgcattcagc caagcagctt gaaccagatt ctcaaaggca gctatgccac cagtccgggc	180
10	aagctgctgg cgtcgggtga ctcgcccatg cgccacgctg aagagacgaa ggccgatgtg	240
	gtggcccccg tcgaaaccag cgtcttcaag ctgcgcacg ccgctgacg catggccgcg	300
	cgctatcgca acttcgcggt gttcacgggt tacgtgggca cgggcaagac ctttgccatc	360
15	aagcaatacg tggcatcgca tccgaacacg cacctgattg aggccacgcc caccatgacg	420
	ccgcagagcc ttgtgcgtct gctggcccg gtggtggccg gctatgacgg caagggcagc	480
	attgatgaca agttccgctc cgtggtgacg gcgcttcgca acaccgacag tctgctcatc	540
20	gtggatgagg ccgaaacgct cagccgcac cagctccaca cgtgcgccg cctccgtgat	600
	ctagccaacg tcggcatcgt gctctgcggc accgaacacc tgtcgggcct catcaagccg	660
	ctgcatggcc agtttgacca aatccgctcg cgcacgggt tctggcccg gacggtgcgc	720
25	gcatcaacc tggagacgc cgcgcctcg gtgcaagccg gcttcggcac ggaagacgta	780
	cctgaagagg tggtgcaacg cctttaccaa tactgcaagg gcagcgcgcg gatgctggtg	840
	gaggggttgg tcgctggcat caaggagttc cgcgctgggc gtccgctgga cgtgaagctg	900
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<212> DNA

<213> *Ralstonia solanacearum*

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	gtggtgctga cgtggaatcg cgcagccatc aagcacggca agcgtgccta cagcaccccc	180
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	attgacgcca tcgcgcccaa ccgcctggaa gaaaagcgcc aggcgcgcgc cgcgatgcc	360
	atcaagcgcc tgcaacaaaa gatggacgag cagaaggccc gtgcgggcat ggtgctcgat	420
50	gtggacagcg tggccgacgg cgtgctgcct gcaatcgagg tggagcccc cctggtggac	480
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	acggaagacg	gtgtgacgca gaacaacgcg gtggcgctgc tgctggagcg cggcgaggcc	240
15	ggcagcctgc	ccagccactt cgccatggcg ctggctggcg ccgccaaggc aggccggaag	300
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	gcgctgctgc	ccgaccacaa gggccgcgtg gtggaggccg ccggctggtg ggggcccgcg	420
20	ctggagtatt	tcaacgcgcc cggcaaacca gatatggccg ctgtacatcg ccgcctggcg	480
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25	aaggcataca	tccgccgctc gaccgaaaac gccttgcccg gcgatgtgta tgtggccgac	660
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	tacgaagcca	tgcgcacgcg gaagtttgtg gctcaactgc cgcaagacga actggaaggc	480
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40 ccgccgaaag ccagctatct ggccgcaata gcgatcctgg gcgttgatgt ggcttatgtg 180

ataacggggg agcgggcgct taatacggcg cgcacgccga tggaaagttgc actgctggag 240

aactaccggc acagcccagc agaggtgcag cgggggggta gcatgcttct cgcacaaacc 300

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Claims

1. A method for the detection of *Ralstonia solanacearum* race 3 biovar 2 strains in a medium, comprising the determination of the presence or the absence in a sample of the medium, of:

- (i) at least one first nucleic acid target having a sequence selected from the group constituted of SEQ ID NO: 1-49, complementary sequences thereof, and homologous sequences thereof, or
 (ii) at least one fragment of said first nucleic acid target, wherein said fragment is not constituted of or comprised in a sequence selected from the group constituted of SEQ ID NO: 111-140;

whereby, if said first nucleic acid target or fragment thereof is present in the sample, it is determined that *Ralstonia solanacearum* race 3 biovar 2 strain is present in the medium.

2. The method according to claim 1, wherein the medium is selected from the group constituted of a potato tissue, a tomato tissue, and a geranium tissue.

3. The method according to claim 1 or 2, wherein the medium is a potato tissue.

4. The method according to claim 3, wherein the potato tissue is the tuber.

5. The method according to any of claims 1 to 4, wherein the sample is obtained from the medium by a nucleic acid extraction.

6. The method according to any of claims 1 to 5, wherein the determination comprises at least one step of hybridization of the nucleic acid target or fragment thereof with a probe or a primer.

7. The method according to claim 6, wherein the probe or primer is a fragment of nucleic acid having a sequence selected from the group constituted of SEQ ID NO: 1-49, homologous sequences thereof, and complementary sequences thereof.

8. The method according to claim 6 or 7, wherein the probe or primer is selected from the group constituted of SEQ ID NO: 141-386.

9. The method according to any of claims 1 to 8, wherein the determination comprises at least one step of nucleic acid amplification.

10. The method according to any of claims 6 to 9, wherein the determination is implemented by a method selected from PCR and NASBA.

11. The method according to any of claims 6 to 8, wherein the determination is implemented by a method selected from Southern blotting, Northern blotting, dot blots, and nucleic acid micro or macro-array hybridization.

12. The method according to any of claims 1 to 11, comprising the determination of the presence or the absence in a sample of the medium, of:

- (i) at least one second nucleic acid target having a sequence selected from the group constituted of SEQ ID NO: 111-140, complementary sequences thereof, and homologous sequences thereof, or
 (ii) at least one fragment of said second target nucleic acid.

13. A nucleic acid having a sequence selected from the group constituted of SEQ ID NO: 1-22, SEQ ID NO: 50-110, SEQ ID NO: 111-140, SEQ ID NO: 141-246, and SEQ ID NO: 247-386 and their complementary sequences.

14. A nucleic acid micro or macro-array comprising a plurality of nucleic acid probes arranged onto a solid support, wherein the nucleic acid probes are fragments of nucleic acids having sequences selected from the group constituted of SEQ ID NO: 1-49, complementary sequences thereof, and homologous sequences thereof, provided that at least one of the nucleic acid probes is not a fragment of a nucleic acid having a sequence selected from the group consisting of SEQ ID NO: 111-140.

15. The nucleic acid micro or macro-array according to claim 14, wherein the nucleic acid probes comprise SEQ ID NO: 247-386.

16. A kit intended for the detection of *Ralstonia solanacearum* race 3 biovar 2 in a medium, comprising at least:

- two primers suitable to amplify a portion of a nucleic acid having a sequence selected from the group constituted of SEQ ID NO: 1-49 and complementary sequences thereof, provided that said portion is not comprised in a nucleic acid having a sequence selected from the group consisting of SEQ ID NO: 111-140 and complementary sequences thereof;

- optionally one detectable nucleic acid probe suitable to hybridize to said amplified portion.

17. The use of:

(i) at least one first nucleic acid target having a sequence selected from the group constituted of SEQ ID NO: 1-49, complementary sequences thereof, and homologous sequences thereof, or

(ii) at least one fragment of said first target nucleic acid, wherein said fragment is not constituted of or comprised in a sequence selected from the group constituted of SEQ ID NO: 111-140;

for detecting *Ralstonia solanacearum* race 3 biovar 2 strains.



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 07 29 1213

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	TEROL J ET AL: "Random genome sequencing of <i>Ralstonia solanacearum</i> strain IVIA 1602 and comparative analysis with strain GMI1000" JOURNAL OF PHYTOPATHOLOGY (BERLIN), vol. 154, no. 9, September 2006 (2006-09), pages 556-564, XP002465338 ISSN: 0931-1785 * the whole document * & DATABASE EMBL [Online] 30 November 2005 (2005-11-30), " <i>Ralstonia solanacearum</i> GSS, clone II122R" retrieved from EBI accession no. EMBL:AJ863704 Database accession no. AJ863704 -----	1,17	INV. C12Q1/68
D,X	GABRIEL DEAN W ET AL: "Identification of open reading frames unique to a select agent: <i>Ralstonia solanacearum</i> race 3 biovar 2" MOLECULAR PLANT-MICROBE INTERACTIONS, vol. 19, no. 1, January 2006 (2006-01), pages 69-79, XP002465339 ISSN: 0894-0282 * the whole document * -----	1	TECHNICAL FIELDS SEARCHED (IPC) C12Q
A	WELLER S A ET AL: "Detection of <i>Ralstonia solanacearum</i> strains with a quantitative, multiplex, real-time, fluorogenic PCR (taqman) assay" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, WASHINGTON,DC, US, vol. 66, no. 7, July 2000 (2000-07), pages 2853-2858, XP002977122 ISSN: 0099-2240 -----		
D,A	WO 2004/042016 A (NASA [US]) 21 May 2004 (2004-05-21) ----- -/--		
3 The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 22 January 2008	Examiner Cornelis, Karen
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EPO FORM 1503 03.08.02 (P04C01)



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 07 29 1213

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	<p>DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; June 2002 (2002-06), MARTINI M ET AL: "Molecular based methods for the detection of Ralstonia solanacearum (race3/biovar2) and for biovar differentiation" XP002465431 Database accession no. PREV200200420663 * abstract * & PHYTOPATHOLOGY, vol. 92, no. 6 Supplement, June 2002 (2002-06), page S52, 2002 ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY; MILWAUKEE, WI, USA; JULY 27-31, 2002 ISSN: 0031-949X</p> <p style="text-align: center;">-----</p>		
			TECHNICAL FIELDS SEARCHED (IPC)
The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 22 January 2008	Examiner Cornelis, Karen
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>& : member of the same patent family, corresponding document</p>			

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Application Number

EP 07 29 1213

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

1-17 (all partially)

☐ The present supplementary European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims (Rule 164 (1) EPC).



The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: Claims 1-17 (all partially)

A method to detect *R. solanacearum* race 3 biovar 2 by detecting SEQ ID NO 1 or a fragment thereof which is not in SEQ ID 111-140, a nucleic acid comprising a sequence as in SEQ ID NO 1 or 50-54, 141-146, 247,248, a micro array with fragments of SEQ ID 1 which do not encompass SEQ IDs 111-140, a kit with 2 primers to amplify a portion of SEQ ID NO 1 which does not correspond to SEQ ID NOs 111-140.

Inventions 2-22: Claims 1-17 (all partially)

A method to detect *R. solanacearum* race 3 biovar 2 by detecting SEQ ID NO 2-22 respectively or a fragment thereof which is not in SEQ ID 111-140, a nucleic acid comprising a sequence as in SEQ ID NO 2-22 respectively or primers and probes according to Tables 2,3,4; a micro array with fragments of SEQ ID 2-22 respectively which do not encompass SEQ IDs 111-140, a kit with 2 primers to amplify a portion of SEQ ID NO 2-22 respectively which does not correspond to SEQ ID NOs 111-140.

3. claims: Claims 1-12,14-17 (all partially)

A method to detect *R. solanacearum* race 3 biovar 2 strain UW551 by detecting SEQ ID NO 23 or a fragment thereof which is not in SEQ ID 111-140, a micro array with fragments of SEQ ID 23 which do not encompass SEQ IDs 111-140, a kit with 2 primers to amplify a portion of SEQ ID NO 23 which does not correspond to SEQ ID NOs 111-140.

Inventions 24-49: Claims 1-12,14-17 (all partially)

A method to detect *R. solanacearum* race 3 biovar 2 strain UW551 by detecting SEQ ID NO 24-49 respectively or a fragment thereof which is not in SEQ ID 111-140, a micro array with fragments of SEQ ID 24-49 respectively which do not encompass SEQ IDs 111-140, a kit with 2 primers to amplify a portion of SEQ ID NO 24-49 which does not correspond to SEQ ID NOs 111-140.

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 07 29 1213

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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22-01-2008

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004042016 A	21-05-2004	AU 2003287336 A1 US 2004086865 A1	07-06-2004 06-05-2004

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

REFERENCES CITED IN THE DESCRIPTION

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